UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte RAFFAELE DE FRANCESCO, LICIA TOMEI, and SVEN-ERIK BEHRENS

Appeal 2007-3260
Application 10/085,476
Technology Center 1600

Decided: November 14, 2007

Before DONALD E. ADAMS, ERIC GRIMES, and MELANIE L. McCOLLUM, *Administrative Patent Judges*.

McCOLLUM, *Administrative Patent Judge*.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 involving claims to a method for identifying an HCV polymerase inhibitor. We have jurisdiction under 35 U.S.C. § 6(b). We affirm.

BACKGROUND

According to Appellants' Specification, "HCV is an enveloped virus containing an RNA positive genome of approximately 9.4 kb," which "has

recently been mapped" (Specification 1). "The majority of the HCV genome is occupied by an open reading frame (ORF) that . . . codes for a single viral polyprotein," which includes both structural and non-structural proteins (*id.* at 2). The non-structural region includes a gene that "codes for two proteins, NS5A (p56) and NS5B (p65)" (*id.*).

The Specification also states that "the replication of HCV is thought to proceed *via* the initial synthesis of a complementary (-)-RNA strand, which serves, in turn, as template for the production of progeny (+)-strand RNA molecules," and that an "RNA-dependent RNA polymerase (RdRp) has been postulated to be involved in both these steps" (*id.* at 3). In addition, the Specification states that an "amino acid sequence present in all the RNA-dependent RNA polymerases can be recognized within the NS5 region," suggesting that "the NS5 region contains components of the viral replication machinery" (*id.*).

The Specification refers to "a method for reproducing in vitro the RNA-dependent RNA polymerase activity of HCV that makes use of sequences contained in the HCV NS5B protein" (*id.*). In particular, the Specification refers to "an enzymatic assay capable of identifying, for therapeutic purposes, compounds that inhibit the enzymatic activities associated with NS5B, including inhibitors of the RdRp" (*id.* at 4).

The Specification states that the "method takes advantage of the fact that the proteins containing sequences of NS5B can be expressed in either eukaryotic or prokaryotic heterologous systems" (*id.*). In addition, "the recombinant proteins containing sequences of NS5B, either purified to apparent homogeneity or present in extracts of overproducing organisms,

can catalyse the addition of ribonucleotides to the 3'-termini of exogenous RNA molecules, . . . in a template-dependent (RdRp) . . . fashion" (*id.*).

DISCUSSION

1. CLAIMS

Claims 12, 14, 17, 18, 22, and 23 are on appeal. Claims 20 and 21 are also pending but have been indicated to be allowed.

The appealed claims have been argued in four groups. The claims within each group stand or fall together. 37 C.F.R. § 41.37(c)(1)(vii). We will focus on claims 12, 14, 22, and 23, which are representative and read as follows:

> 12. A method for identifying a HCV RNA-dependent RNA polymerase inhibitor comprising:
>
> (a) incubating *in vitro* a composition comprising a purified HCV NS5B recombinant protein, ribonucleotide substrates, an RNA template, and a test compound, under conditions suitable to produce NS5B RNA-dependent RNA polymerase activity in the absence of said compound, wherein said recombinant protein was expressed in either a eukaryotic or prokaryotic heterologous system and purified to apparent homogeneity; and
>
> (b) measuring the ability of said compound to affect said NS5B RNA-dependent RNA polymerase activity.
>
> 14. The method of claim 12, wherein said method measures primer independent RNA-dependent RNA polymerase activity.
>
> 22. A method for identifying a HCV RNA-dependent RNA polymerase inhibitor comprising:
>
> (a) incubating *in vitro* a composition comprising HCV NS5B, ribonucleotide substrates, an RNA template, and a test compound, under conditions suitable to produce NS5B RNA-dependent RNA

> polymerase activity in the absence of said test compound, wherein said HCV NS5B was expressed in either a eukaryotic or prokaryotic heterologous system; and
>
> (b) measuring the ability of said test compound to affect said NS5B RNA-dependent RNA polymerase activity.
>
> 23. The method of claim 22, wherein said method measures primer independent RNA-dependent RNA polymerase activity.

Thus, claims 12 and 22 are each directed to a method for identifying an HCV RNA-dependent RNA polymerase inhibitor comprising incubating *in vitro* a composition comprising HCV NS5B protein, ribonucleotide substrates, an RNA template, and a test compound under conditions suitable to produce NS5B RNA-dependent RNA polymerase activity in the absence of the test compound; and measuring the ability of the test compound to affect the NS5B RNA-dependent RNA polymerase activity. Claims 12 and 22 each also recites that the HCV NS5B protein was expressed in either a eukaryotic or prokaryotic heterologous system. In addition, claim 12 states that the HCV NS5B protein was purified to apparent homogeneity.

Claims 14 and 23 depend from claims 12 and 22, respectively, and each recites that the method measures primer independent RNA-dependent RNA polymerase activity. Claims 14 and 23 do not recite that only primer independent RNA-dependent RNA polymerase activity is measured. Thus, giving claims 14 and 23 their broadest reasonable interpretation, we interpret these claims to include methods in which only primer independent RNA-dependent RNA polymerase activity is measured, as well as methods in which the measured RNA-dependent RNA polymerase activity includes

both primer independent and primer dependent RNA-dependent RNA polymerase activity.

2. REFERENCES

The Examiner relies on the following reference:

Tomei et al. (Tomei) "NS3 Is a Serine Protease Required for Processing of Hepatitis C Virus Polyprotein," *Journal of Virology*, Vol. 67, pp. 4017-4026 (1993)

Appellants rely on the following references:

Hagedorn et al. US 5,981,247 Nov. 09, 1999

Miller et al. (Miller) "Hepatitis C Virus Shares Amino Acid Sequence Similarity with Pestiviruses and Flaviviruses as well as Members of two Plant Virus Supergroups," *Proc. Natl. Acad. Sci.*, Vol. 87, pp. 2057-2061 (1990)

Chung et al. (Chung) "Identification and Characterization of a Hepatitis C Virus-Specific RNA-Dependent RNA Polymerase Activity from Extracts of Infected Liver Tissue," *Hepatology*, Vol. 16, p. 132A (1992)

Grakoui et al. (Grakoui) "Expression and Identification of Hepatitis C Virus Polyprotein Cleavage Products," *Journal of Virology*, Vol. 67, pp. 1385-1395 (1993)

Tsutsumi et al. (Tsutsumi) "Detection of Antigens Related to Hepatitis C Virus RNA Encoding the NS5 Region in the Livers of Patient with Chronic Type C Hepatitis," *Hepatology*, Vol. 19, pp. 265-272 (1994)

3. OBVIOUSNESS

Claims 12, 14, 17, 18, 22, and 23 stand rejected under 35 U.S.C. § 103 as obvious in view of Tomei. Tomei describes HCV as possessing a positive-sense RNA genome encoding a large polyprotein and that a portion

of this polyprotein, which has been referred to as NS5, "contains a GDD consensus sequence found in several viral RNA-dependent RNA polymerases, suggesting that it may be involved in viral replication" (Tomei 4017). Tomei discloses that HCV polyprotein expression was examined "by using the vaccinia virus T7 transient expression system in transfected cells" (*id.*). Specifically, HCV cDNA containing the entire HCV open reading frame was introduced into a plasmid vector and used for transfection experiments in HeLa cells (*id.* at 4018 and 4020).

Tomei also discloses that the cell lysates of the transfected cells were immunoprecipitated with region-specific antisera and "[t]wo proteins of 56 and 65 kDa were immunoprecipitated with antisera specific for . . . the NS5 gene" (*id.* at 4020-4021). "The N-terminal protein was designated NS5a, whereas the C-terminal protein was called NS5b" (*id.* at 4021). Tomei states that the "data suggest that apparently authentic processing of the entire HCV polyprotein can occur in this test system" (*id.*). Tomei also discloses that the "GDD consensus sequence characteristic of RNA-dependent RNA polymerases is located in NS5b . . . , indicating that this protein may act as a viral RNA replicase during HCV-specific RNA synthesis" (*id.* at 4024).

The Examiner finds that Tomei teaches "DNA constructs and transient expression of the HCV genome and characterize[s] the post-translational processing of the HCV transcript, and specifically transcribe[s] and translate[s] NS5B, described by SEQ ID NO: 1, in a eukaryotic heterologous system" (Answer 6). The Examiner also finds that one of ordinary skill in the art

> would have been . . . motivated to incubate together the HCV NS5B protein, ribonucleotide substrates and a RNA template

> under conditions suitable to produce RNA-dependent RNA polymerase activity, wherein said incubation takes place *in vitro* in the presence of potential target molecules which may inhibit the action of the NS5B protein as a means of identifying potential therapeutics to be used against the NS5B protein and HCV.

(*Id.* at 4-5.) We agree with the Examiner that Tomei supports a prima facie case of obviousness.

Appellants do not dispute that it would have been obvious to incubate *in vitro* an HCV NS5B protein, ribonucleotide substrates, a RNA template, and a test compound under conditions suitable to produce RNA-dependent RNA polymerase activity in the absence of the test compound, as a means of identifying potential therapeutics to be used against the NS5B protein and HCV. Instead, Appellants argue that the "method of claim 22 distinguishes Tomei et al. by . . . employing HCV NS5B expressed in either a eukaryotic or prokaryotic heterologous system to identify a RNA-dependent RNA polymerase inhibitor" (Br. 7).

In particular, Appellants argue that "[p]rior to the present application there was significant uncertainty concerning the relevance of HCV NS5B produced in recombinant expression systems, such as that employed by Tomei et al., to a naturally produced HCV protein product" (Br. 9). The uncertainties "are reflected in the differences between published results obtained from HCV infected liver cell versus recombinantly expressed HCV polyprotein. The uncertainties are also noted in cautionary language used in publications concerning recombinantly produced NS5B." (Br. 9-10.) In support of this position, Appellants rely on Grakoui and Tsutsumi, as well as on language in Tomei (Br. 10-11). Appellants argue that none of Tomei,

Grakoui, and Tsutsumi "provide[s] results demonstrating that an observed protein provides HCV RNA-dependent RNA polymerase activity. Thus, the present application, not the prior art, resolves the scientific uncertainties concerning the relevance of NS5B to providing RNA-dependent RNA polymerase activity." (Br. 11.)

We are not persuaded by these arguments. Although Tomei does not present data demonstrating that the NS5B protein has RNA-dependent RNA polymerase activity, Tomei does state that the "data suggest that apparently authentic processing of the entire HCV polyprotein can occur in this test system" (Tomei 4021). In addition, Tomei discloses that the "GDD consensus sequence characteristic of RNA-dependent RNA polymerases is located in NS5b . . . , indicating that this protein may act as a viral RNA replicase during HCV-specific RNA synthesis" (*id.* at 4024). Thus, even though Tomei includes cautionary language typical of a scientific publication, such as stating that "this protein *may* act as a viral RNA replicase" (emphasis added) and, in its conclusion, stating that "the results . . . may not faithfully reproduce the proteolytic events which take place during HCV infection," we conclude that Tomei supports a prima facie case that NS5B protein having RNA-dependent RNA polymerase activity can be expressed in a eukaryotic or prokaryotic heterologous system.

In addition, we do not agree with Appellants that Tsutsumi and Grakoui provide sufficient evidence to rebut this prima facie case. Grakoui, which was published a few months before Tomei, indicates that "HCV polypeptides appear to be produced by translation of a long open reading frame and subsequent proteolytic processing of this polyprotein" and that

this "polyprotein probably encodes nonstructural proteins including . . . NS5A (58 kDa), and NS5B (68 kDa)." (Grakoui 1385). As pointed out by Appellants (Br. 11), Grakoui, in its concluding paragraph, states that the experiments described in the article provide "a preliminary picture of HCV polyprotein organization" and that "this view is far from complete, and additional studies are needed to define polyprotein cleavage sites . . . and to verify that the products observed in these expression studies are similar to those produced in authentic HCV infections" (Grakoui 1393). However, we do not agree that these statements provide persuasive evidence that the NS5B protein described in Tomei is not an authentic protein having RNA-dependent RNA polymerase activity. In fact, Grakoui corroborates that the NS5 region of the HCV polyprotein forms two proteins, NS5A and NS5B, having the approximate sizes described in Tomei – 56 and 65 kDa, respectively.

Tsutsumi describes an HCV-NS5-related antigen detected in human liver having a molecular size of 86 kD (Tsutsumi 269-270). Tsutsumi states that this antigen is "slightly larger" than the size of NS5B described in Grakoui, noting that Tsutsumi "observed the products derived from native HCV, whereas Grakoui . . . observed the polypeptide expressed from HCV cDNA in vaccinia virus (*id.* at 270). Appellants argue that the "magnitude of the different molecular weights for the infected human liver and recombinantly processed HCV NS5 regions point to different proteins and not minor variations" (Br. 10). However, we do not agree that the larger size described in Tsutsumi provides persuasive evidence that the NS5B protein described in Tomei is not an authentic protein having RNA-dependent RNA

polymerase activity. In fact, Tsutsumi itself speculates that the size "discrepancy may have resulted in different host cells" (Tsutsumi 270).

Appellants also argue that "[a]dditional considerations illustrating the inventive nature of the claimed assay include: (1) apparent failure and difficulty encountered by others in demonstrating the HCV region responsible for RNA-dependent RNA polymerase; and (2) a long-felt need for an HCV RNA-dependent RNA polymerase assay to look for polymerase inhibitors" (Br. 11). Specifically, Appellants argue that Hagedorn states that "no polymerase activity has been detected for HCV p68," encoded by the NS5B region, as described in a 1991 article by Koonin, and that "[p]rior attempts . . . to express the NS5B coding region as a fusion protein, using existing expression systems that facilitate purification of the fusion product and specific cleavage have failed to yield any active polymerase" (Br. 12, citing Hagedorn, col. 1, l. 59, to col. 2, l. 7).

In addition, Appellants argue that the "long-felt need is evident based on the medical importance of HCV, the desirability of an assay to screen for a HCV RNA-dependent RNA polymerase inhibitor, and the time difference between prior art speculations concerning HCV RNA-dependent RNA polymerase and [Appellants'] priority application" (Br. 12). Specifically, Appellants argue that, as shown in Miller, "[s]peculations concerning the HCV protein responsible for RNA-dependent RNA polymerase are noted at least as early as 1990," which is five years before the filing date of Appellants' priority application (*id.*).

We are not persuaded by this argument. Miller, which describes the HCV polyprotein sequence as having a "putative replicase" (Miller 2061),

was published five years before the priority date of Appellants' application. However, Appellants have not provided any evidence that there was a persistent need over this five year period for a method for identifying an HCV RNA-dependent RNA polymerase inhibitor. "[L]ong-felt need is analyzed as of the date of an articulated identified problem and *evidence* of efforts to solve that problem." *Texas Instruments, Inc. v. United States Intl. Trade Comm.*, 988 F.2d 1165, 1178 (Fed. Cir. 1993) (emphasis added). Appellants have provided some evidence of unsuccessful attempts to express the NS5B coding region as a fusion product having active polymerase. However, Appellants have not shown that this need was not satisfied by Tomei. Therefore, we do not agree that these unsuccessful attempts overcome the Examiner's prima facie case that claim 22 would have been obvious over Tomei. *See Newell Companies v. Kenney Mfg. Co.*, 864 F.2d 757, 768 (Fed. Cir. 1988) ("once another supplied the key element, there was no long-felt need or, indeed, a problem to be solved").

We conclude that the Examiner has set forth a prima facie case that claim 22 would have been obvious over Tomei, which Appellants have not rebutted. We therefore affirm the rejection of claim 22 under 35 U.S.C. § 103.

With regard to claim 12, Appellants additionally argue that the "[r]eference to purified to apparent homogeneity further distinguishes Tomei et al. by indicating a very high degree of purity" (Br. 14). In support of this position, Appellants argue that Hagedorn and Chung "point to prior art difficulties in obtaining purified HCV RNA-depend[e]nt RNA polymerase" (*id.*).

We are not persuaded by this argument. Claim 12 recites "incubating *in vitro* a composition comprising a purified HCV NS5B recombinant protein." We agree with the Examiner that "by virtue of [the comprising] language any limitation of purity associated with the referred to NS5B is removed" (Answer 13).

We conclude that the Examiner has set forth a prima facie case that claim 12 would have been obvious over Tomei, which Appellants have not rebutted. We therefore affirm the rejection of claim 12 under 35 U.S.C. § 103. Claims 17 and 18 fall with claim 12.

With regard to claims 14 and 23, the Examiner argues that "the obvious methods of assaying NS5B for RNA-dependent RNA polymerase activity . . . would inherently also measure primer independent RNA-dependent RNA polymerase activity" (Answer 12 and 14). Specifically, the Examiner argues:

> In fact any measure of RNA-dependent RNA polymerase activity will measure such activity that is primer independent. This is in contrast to primer dependent RNA-dependent RNA polymerase activity, which would only be measured in the presence of a primer. Thus . . . , the claims which appellants appear to believe are further limiting, and refer to primer independent RNA-dependent RNA polymerase activity, in fact are obvious for the same reasons that the base [claims 12 and 22 are] obvious.

(*Id.*) We conclude that the Examiner has set forth a prima facie case of inherency.

Appellants argue that Tomei "fails to even demonstrate that NS5B provides for RNA-dependent RNA polymerase activity. Absent knowing that NS5B provides for RNA-dependent RNA polymerase activity the

skilled artisan would not be motivated to further characterize the enzyme, or set up an assay to look for inhibitors by measuring primer independent RNA-dependent RNA polymerase activity." (Br. 14-15.)

We are not persuaded by this argument. We agree that Tomei does not present data demonstrating that NS5B provides for RNA-dependent RNA polymerase activity, much less that this activity is primer independent. However, the Specification discloses that extracts of cells infected with a vector containing the cDNA region corresponding to the NS5B protein (pBac5B) contain an "enzymatic activity that catalyses *de novo* RNA synthesis" and that this "activity was shown to be . . . independent of an added primer" (Specification 10-11 and 6).

In addition, Appellants have not presented persuasive evidence that measuring primer independent RNA-dependent RNA polymerase activity would not be inherent in the methods of base claims 12 and 22. Thus, Appellants have not overcome the Examiner's prima facie case that the methods of claims 14 and 23 would have been inherent in the methods of claims 12 and 22, respectively, and that therefore claims 14 and 23 would have been obvious over Tomei for the same reasons as claims 12 and 22. As a result, we affirm the rejection of claims 14 and 23 under 35 U.S.C. § 103.

SUMMARY

The Examiner's position is supported by the preponderance of the evidence of record. We therefore affirm the rejection of claims 12, 14, 17, 18, 22, and 23 under 35 U.S.C. § 103.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

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Merck and Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907